Biochimica et Biophysica Acta, 1106 (1992) 257–263

BBAMEM 75619

Uptake of L-leucine and L-phenylalanine across the basolateral cell surface in isolated oxyntic glands

Luis Sobrevía [1], Vivian Medina, Karin Reinicke and Iván Bravo

Department of Physiological Sciences, Faculty of Biological Sciences and Natural Resources, University of Concepción, Concepción (Chile)

(Received 12 February 1992)

Key words: Leucine; Phenylalanine; Amino acid transport; Basolateral membrane

The time course kinetics, specificity and sodium-dependence of L-leucine and L-phenylalanine uptake by rabbit isolated oxyntic glands were studied in order to identify the systems involved in the transport of branched-chain and aromatic neutral amino acids through the basolateral cell membrane. The uptake was measured directly in the disrupted cells after incubation of the glands with the ^{3}H-labelled amino acid both in a sodium-containing and a sodium-free medium. The uptake of L-leucine was largely carrier-mediated whilst L-phenylalanine was taken up by either carrier-mediated and nonsaturable processes. Both amino acids were taken up by a Na^+-independent process. The kinetic parameters of L-leucine and L-phenylalanine carrier-mediated influx were, respectively: $K_t = 2.71$ mM and $J_{max} = 1390$ nmol mg^{-1} s^{-1}, $K_t = 1.03$ mM and $J_{max} = 176$ nmol mg^{-1} s^{-1}. From cross-inhibition studies it can be inferred that L-leucine is primarily transported by a Na^+-independent system which shows specificity for bulky side chains dipolar amino acids. The system displays similar affinities for L-phenylalanine ($K_i = 2.81$ mM) and L-isoleucine ($K_i = 2.62$ mM). Similar results were obtained from self-inhibition experiments: the K_i of the carrier-mediated uptake of L-leucine and L-phenylalanine were 2.12 and 2.40 mM (from a Hanes plot) or 3.2 and 0.8 mM (from a Dixon plot), respectively. It is concluded that a sodium-independent transport system, like Christensen's 'L' type, is shared by branched-chain and aromatic dipolar amino acids, which only shows slight differences in their affinities for the carrier.

Introduction

Amino acid transport across the basal lateral membrane of polar epithelial cells, such as those of the small intestine [1–4] and accessory digestive organs [5–8], is mediated by carrier systems which are commonly operative in mammalian non-epithelial cells [9–11]. These pathways include Na^+-dependent and Na^+-independent transport systems for neutral amino acids, from which the Christensen's A, ASC and L systems are the more ubiquitous. Mircheff et al. [2] showed that A-, ASC- and L-like amino acids pathways exist in jejunal basal lateral membrane, in which the system L appears to provide the major route for exit of amino acids from the cells into the blood. From the amino acid transport systems which are common to non-epithelial cells [9,10], only sodium-independent systems are present in the brush-border membrane of the intestinal epithelium [4].

Limited information is available about the pathways mediating amino acid transport through the gastric epithelia. Recent reports [12–14] show that amino acids are transported from the lumen into the cells of the mammalian stomach and suggest that the process is carrier-mediated. A considerable fraction of the amino acid taken up by gastric epithelial cells can be absorbed [12,14], suggesting that, in addition of its known oxyntic and peptic secretory functions, the gastric epithelium may play a role in the absorption of nutrients. On the other hand, it has been shown that intrarterially injected L-amino acids are transported across the blood-tissue interface of the stomach at rates which are greater for acidic than for neutral and basic amino acids [15]. Other results suggest that some of the classical transport systems available to amino acids in non-epithelial cells [9–11], such as the L system, are also operative at the blood-tissue interface of the canine gastric wall [16]. In a recent report [17] a Na^+-dependent amino acid transport system, similar to the Christensen's A transporter was characterized in the basal lateral surface of isolated oxyntic glands.

[1] Present address: Department of Physiology and Biophysics, Faculty of Medicine, University of Chile, P.O. Box 70005, Santiago, Chile.

Correspondence: I. Bravo, Depto. de Ciencias Fisiológicas, Universidad de Concepción, Casilla 2407, Apartado 10, Concepción, Chile.

In a variety of cell types, transport of branched chain and aromatic amino acids is mediated by system L [18] which shows high exchange properties [19], being specially relevant for the absorption of amino acids across the intestinal epithelium [4]. A similar role should be valid for the L system at the basolateral side of the gastric epithelium, since absorption of leucine and phenylalanine appears to occur in this barrier [12–14]. The participation of L-system in the transport of blood-borne substrates for the nutrition of the gastric epithelial cells may also be important, because branched-chain amino acids which are taken up by the stomach 'in vitro' seem to be transformed to the corresponding 2-oxoacid [20,21], and leucine is oxidized by the gastric mucosa 'in vitro' [21].

The aim of the present study was to investigate if system L is present at the blood side of the gastric epithelium. Studies on kinetics, sodium-dependence and cross-inhibition of L-leucine and L-phenylalanine uptake indicate that a L-like transport system is in fact operative at the basolateral surface of isolated oxyntic glands. Preliminary results of this work have been published previously [22,23].

Materials and Methods

Isolation of the oxyntic glands

Rabbits (1.2–2.0 kg) were used for the experiments. By following the method described by Berglindh and Öbrink [24], the gastric glands were isolated from the corpus of the non-stimulated stomach in the anesthetized (30 mg/kg Nembutal) animals. After performing subdiaphragmatic ligatures of the aorta and mesenteric vessels, the stomach was perfused with a saline solution (154 mM NaCl, 10 mM KCl) under high pressure through an aortic cannula introduced in a retrograde direction. When the stomach appeared totally clear of blood it was rapidly removed, the lesser curvature cut open and rinsed in saline solution. The mucosa (7–8 g) of the corpus was striped off, minced into small pieces and transferred to a 200 ml flask containing 25 ml of collagenase solution. This solution consisted of 1 mg collagenase Type IV (from *Clostridium histolyticum*, Sigma Chem. Co) dissolved in 1 ml incubation medium (IM), with the following composition (mM): NaCl, 132 + 4; KCl, 10; $CaCl_2$, 1; $MgCl_2$, 0.8; Na_2HPO_4, 5; NaH_2PO_4, 1.2; pyruvic acid, 1; glucose, 11.1 and bovine serum albumin, 1 mg/ml (pH 7.4). The flask was aerated with 100% oxygen, sealed and incubated at 37°C and continuously stirred for 40 min. The glandular suspension was filtered through a nylon cloth (pore size 230 μm) into 15-ml test tubes with conical bottoms. The gastric glands were separated from isolated cells by sedimentation and repeated washing. The yield of oxyntic glands amounted to approx. 7 mg wet weight corresponding to a dry weight of 1 mg.

The viability of the isolated oxyntic glands was determined by the trypan blue dye technique as described elsewhere [17]. About 95% of the glands excluded the dye. In each of the tinged gland, only one to two cells were unable to exclude the Trypan blue. The integrity of the gastric glands and oxyntic cells was determined by using both light and electron microscopy [17]. The dependence of amino acid transport upon extracellular sodium was studied by measuring the uptake of tritium-labelled amino acids ([^{3}H]aa) by the isolated glands incubated with the IM in which sodium was iso-osmotically replaced with choline (as choline chloride). Inhibition experiments were performed by measuring the uptake of a [^{3}H]aa in the presence and absence of unlabelled amino acids at concentrations which ranged from 1 to 100 mM.

Measurement of amino acid uptake

A reaction mixture, previously aerated with 100% oxygen, was incubated at 37°C in 1.5 ml Eppendorf tubes with constant and gentle swirling. In each tube the reaction mixture contained: the isolated glands (equivalent to 1 mg dry weight) suspended in 1 ml of incubation medium and L-[^{3}H]leucine (at a final concentration of 40.8 μM) or L-[^{3}H]phenylalanine (at a final concentration of 33.3 μM). At indicated time intervals (15 to 600 s for leucine, and 15 to 180 s for phenylalanine) the mixtures were removed and immediately centrifuged at 11 630 × g (Microfuge, Heraus) during 15 s. The pellets obtained were prepared for counting as was described previously [17]. The cellular uptake (U%) of the labelled amino acid was calculated as [cpm[^{3}H] ml^{-1} (sample)/ cpm[^{3}H] ml^{-1} (standard)] × 100, where the standard solution contained 40.8 nmol L-[^{3}H]leucine or 33.3 nmol L-[^{3}H]phenylalanine per ml IM. From the U values and the specific activity of the [^{3}H]aa (50 and 60 Ci/mmol for leucine and phenylalanine, respectively) the cellular uptake was expressed as nmol mg^{-1}. When unlabelled amino acids were added to the test tube, the total influx of leucine or phenylalanine (nmol mg^{-1} s^{-1}) was calculated as (U%/100)·[aa], in which [aa] corresponds to total amino acid concentration in nmol mg^{-1} and the influx time was 1 s.

In order to investigate the possibility that a considerable fraction of a [^{3}H]aa could be distributed in an extracellular compartment, oxyntic glands were incubated with [^{14}C]sucrose for 30 to 600 s and processed for counting. Only 2% of the extracellular marker was detected in the glands. In the kinetic studies of leucine and phenylalanine uptake and when other amino acids were used as competitors, control measurements of [^{3}H]aa uptake were performed in the presence of sucrose at concentrations equivalent to those of unlabelled amino acids. Sucrose showed no appreciable effect on amino acid uptake as in previous experiments

[17]. The kinetic parameters were determined by using Enzfitter program (Elsevier Biosoft). Statistical significance was assessed using a Student's t-test.

Chemicals

The radioactive molecules L-[^{3}H]leucine (50 Ci/mmol), L-[^{3}H]phenylalanine (60 Ci/mmol) and [^{14}C]sucrose (645.4 Ci/mmol) and the liquid scintillation fluors were purchased from New England Nuclear (USA). All other chemicals used were obtained from Merck (Germany) and Sigma Chemical Co. (USA).

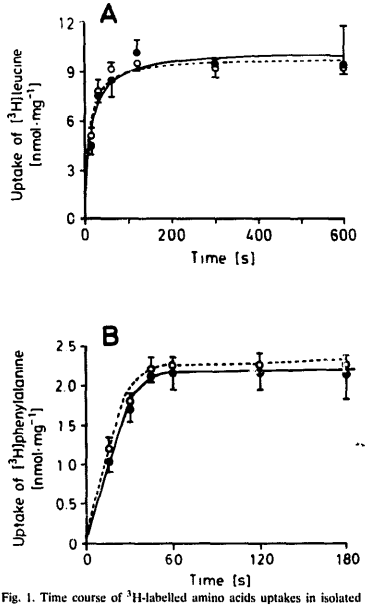

Fig. 1. Time course of ^{3}H-labelled amino acids uptakes in isolated oxyntic glands. (A) The glands were incubated with 40.8 μM L-[^{3}H]leucine in both a normal Na^+-containing (○———○) and a Na^+-free medium (choline chloride, ●———●). The amino acid uptake was measured at indicated times, as described in Materials and Methods. Each point denotes the mean ± S.D. of 10 measurements. Values obtained in the absence of Na^+ are not statistically different from those observed in the normal medium ($P > 0.02$). (B) Uptake time curves of 33.3 μM L-[^{3}H]phenylalanine in a normal sodium-containing medium (○———○) and a sodium-free medium (●———●). Each point is the mean ± S.D. of eight measurements. No significant difference there are between the values obtained in both media ($P > 0.02$).

Results

Uptake time course

Fig. 1A shows the time-dependent uptake of L-[^{3}H]leucine by the isolated oxyntic glands measured both in a normal Na^+-containing and a Na^+-free medium. It is apparent that in the normal medium, cellular uptake of the amino acid reaches a steady state from 120 to 600 s. A similar time course of uptake was observed in the Na^+-free medium. In both cases, leucine uptake was roughly linear up to 30 s and this time was used as the initial velocity of influx [17]. Similar patterns to those displayed in Fig. 1A were observed when measurements were made in the presence of unlabelled leucine (1, 5 and 10 mM). In these experiments the maximal uptake of L-[^{3}H]leucine decreased as the concentration of unlabelled leucine was increased but in all cases the steady-state was reached by about 120 s (results not shown).

The time course for L-[^{3}H]phenylalanine uptake measured in both a Na^+-containing and a Na^+-free medium is shown in Fig. 1B. It is apparent that in either condition the initial velocity of the influx was roughly maintained up to 30 s and the uptake curves reached a plateau about 60 s after incubation.

Kinetics of L-leucine and L-phenylalanine influx

The isolated oxyntic glands were incubated during 30 s with 40.8 μM L-[^{3}H]leucine or 33.3 μM L-[^{3}H]phenylalanine, both in a normal sodium-containing and a sodium-free medium. The cellular uptake of the labelled amino acid was measured in the absence (control condition), and in presence of different concentrations of the respective unlabelled analogue (L-leucine, 1 to 100 mM; L-phenylalanine, 1 to 50 mM). Because of variability in uptake measured in different preparations, the values (U_i) obtained in various concentrations of the unlabelled amino acid were normalized relative to control uptake values (U_c) according to the expression: $(U_i/U_c) \cdot U_{cm}$, in which U_{cm} is the arithmetic mean of the overall control uptake values. Results obtained in normal medium were not statistically different from those measured in Na^+-free medium (data are not shown) which indicated a primarily Na^+-independent uptake of both amino acids. Therefore, the overall uptake values were pooled in a single series from which the total influx of L-leucine (or L-phenylalanine) was obtained as described in Materials and Methods. Corrected values for the non saturable component allowed to obtain the carrier mediated influx of the amino acid (see legend Fig. 2). Total influx values are shown in Fig. 2 (upper curves, panels A and B) which also illustrates that the corresponding influx-concentration curves for the carrier-mediated component (lower curves, panels A and B) of both leucine and phenylalanine uptake followed a rectangular hy-

perbole. The Lineweaver-Burk plots of the data (insets) suggest simple saturation kinetics with the following values (mean ± S.D.): $K_t = 2.71 \pm 0.47$ mM and $J_{max} = 13.90 \pm 0.73$ nmol mg^{-1} s^{-1} for L-leucine and $K_t = 1.03 \pm 0.08$ mM and $J_{max} = 1.76 \pm 0.03$ nmol mg^{-1} s^{-1} for L-phenylalanine.

Inhibition by neutral amino acids

In order to determine the specificity of carrier systems involved in leucine transport, the effect of several neutral L-amino acids on the uptake of L-leucine was assayed. Fig. 3 depicts the results obtained when the uptake of 40.8 μM L-[^{3}H]leucine was measured in the presence of different concentrations of unlabelled amino acids (1 to 75 mM) both in normal and Na^+-free medium. In both conditions no considerable effect was obtained using L-serine or L-alanine, whilst L-cysteine slightly inhibited leucine uptake (16 ± 2% and 14 ± 3% at 75 mM in normal and sodium-free medium, respectively). The other two neutral amino acids L-isoleucine and L-phenylalanine, significantly inhibited leucine uptake. These results suggest that L-leucine uptake is mediated by a Na^+-independent system which prefer dipolar-amino acids with bulky side chains. Because leucine uptake measured in the presence of an inhibitor (e.g. L-isoleucine or L-phenylalanine) in the normal medium was not statistically different to that performed in the sodium-free medium, values obtained in both media were pooled in a single series and processed as was above described to obtain the Na^+-independent carrier-mediated uptake of leucine (Fig. 4). The apparent inhibition constants (K_i) were determined by using the Dixon's graphic analysis for the two inhibitor amino acids (inset in Fig. 4). The apparent K_i values (mean ± S.D.) obtained were 2.62 ± 0.04 mM and 2.81 ± 0.02 mM for L-isoleucine and L-phenylalanine, respectively.

A kinetic analysis of the inhibition of uptake of labelled amino acid induced by the respective unlabelled molecule has been already performed [27–29]. In this case, increasing concentration of the non-radioactive amino acid are regarded as competitively inhibiting the uptake of the radioactive analogue. According to this approach any non-mediated component to substrate uptake will not interfere in the K_i determinations. By following this procedure the K_i of the carrier-mediated L-[^{3}H]leucine uptake for unlabelled leucine was estimated (Fig. 5) The K_i value obtained (2.12 ± 0.06 mM) was lower but in the same range than the half-saturation concentration for leucine ($K_t = 2.71$ mM, Fig. 2).

A kinetic analysis of L-[^{3}H]phenylalanine uptake inhibition by unlabelled phenylalanine was performed according to this procedure and the K_i value was 2.4 ± 0.07 mM. This value is higher but comparable to the K_t value (1.03 mM, Fig. 2) determined for the influx of phenylalanine. The nonsaturable component corrected values for both L-[^{3}H]leucine and L-[^{3}H]phenylalanine uptake were analyzed with a Dixon plot (results not shown). The K_i values obtained for

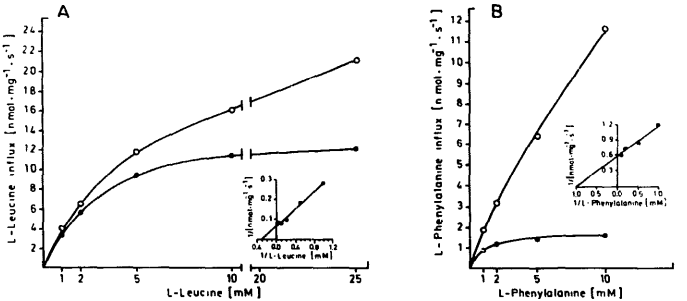

Fig. 2. Kinetics of L-leucine (A) and L-phenylalanine (B). The uptake of 40.8 μM L-leucine or 33.3 μM L-phenylalanine was measured in the absence and in the presence of different concentrations of the respective unlabelled analogue, both in a sodium-containing and a sodium-free medium. The results obtained (not shown) were pooled and processed (see text) in order to calculate the total influx of L-leucine (upper curve, panel A) and L-phenylalanine (upper curve, panel B). The carrier-mediated influx of each amino acid (lower curve, panels A and B) was obtained as the difference between the total influx and the nonsaturable component of substrate influx (J_d). J_d was estimated as [25,26]: $P \cdot [S]$, in which P is the permeability coefficient ($0.425 \cdot 10^{-7}$ l nmol mg^{-1} s^{-1} for L-leucine; $101 \cdot 10^{-6}$ l nmol mg^{-1} s^{-1} for L-phenylalanine) and [S] is substrate concentration. P was determined by measuring total influx at maximal [S]: 100 mM L-leucine and 50 mM L-phenylalanine. The insets illustrate a Lineweaver-Burk plot of the corresponding data to carrier-mediated influx. Values obtained (mean ± S.D.) were: $K_t = 2.71 \pm 0.47$ mM and $J_{max} = 13.90 \pm 0.73$ nmol mg^{-1} s^{-1} for L-leucine, and $K_t = 1.03 \pm 0.08$ mM and $J_{max} = 1.76 \pm 0.03$ nmol mg^{-1} s^{-1} for L-phenylalanine.

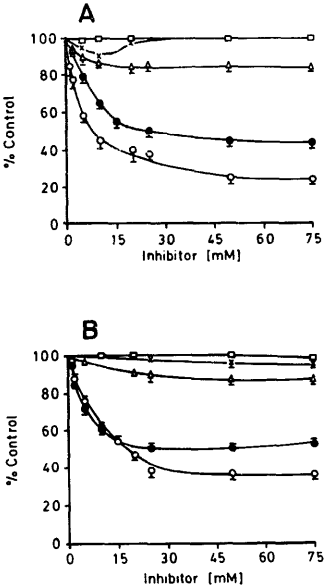

Fig. 3. Effect of neutral L-amino acids on L-[^{3}H]leucine uptake, both in a normal sodium-containing (A) and a sodium-free (B) medium. Values were normalized in relation to the control uptake, i.e., to that measured in the absence of potential inhibitors. Each point denotes the mean ± S.D. of 6–12 measurements. The symbols are: isoleucine (○), phenylalanine (●), cysteine (△), alanine (□) and serine (×). Only isoleucine and phenylalanine significantly inhibited ($P < 0.001$ at ≥ 5 mM) the L-leucine uptake in both media. Measurements performed in the sodium-free medium were not statistically different ($P > 0.01$) to those performed in the normal medium.

L-leucine and L-phenylalanine (as inhibitors of the carrier-mediated uptake of the respective labelled analogue) were 3.2 and 0.8 mM, respectively.

Discussion

Berglindh and colleagues [24,30] have shown that the gastric glands isolated from the corpus of the rabbit stomach are appropriate for studying the function of gastric epithelial cells in a state closely resembling 'in vivo' conditions. Because isolated oxyntic glands maintain the polarity of both cell sides and the basolateral surface of the glands is more accessible than the lumen

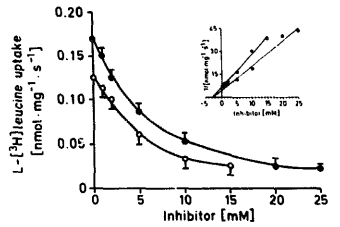

Fig. 4. Inhibition of L-leucine uptake by L-isoleucine (○; 1 to 25 mM) or L-phenylalanine (●; 1 to 15 mM). The uptake of 40.8 μM L-[^{3}H]leucine was measured in the absence (control) and the presence of the inhibitors, both in a sodium-containing and a sodium-free medium. The data obtained were pooled in a single series and processed as described in Fig. 2 in order to calculate the carrier-mediated uptake of L-leucine. Each value gives the mean ± S.D. of 12–24 determinations. The inset shows a Dixon plot of the carrier-mediated uptake of leucine at indicated concentrations of the inhibitors, and the K_i values (mean ± S.D.) were 2.62 ± 0.04 and 2.81 ± 0.02 mM for L-isoleucine and L-phenylalanine, respectively.

[30], this model was selected in our laboratory to study the characteristics of the pathways involved in the basolateral transport of amino acids. In a previous report [17] using viable oxyntic glands we provided evidence that a transport system which shows Na^+-dependence, tolerance of *N*-methyl group and specificity by short-chain amino acids, like the Christensen's system A [9,10], is present at the basolateral surface of the gland cells. In the present study, by measuring the specificity of L-leucine uptake and the kinetic constants

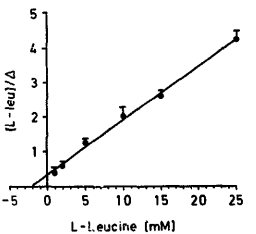

Fig. 5. Hanes plot of L-leucine inhibition of L-[^{3}H]leucine uptake. The uptake of the radioactive substrate was measured in the absence (*U* (control)) and the presence (*U*) of unlabelled leucine (1 to 25 mM) as was previously described in Fig. 2. Δ is the inhibitable portion of leucine uptake of each concentration of leucine used and was obtained as *U* (control) minus U_i; [L-Leu] is the unlabelled L-leucine concentration (mM). Each point denotes the mean ± S.D. of 8–10 determinations. The affinity of leucine for its carrier was calculated assuming $K_i = K_t$ [19] and the value obtained (mean ± S.D.) was 2.12 ± 0.06 mM.

of L-leucine and L-phenylalanine transport through the basolateral side of the oxyntic glands, we have characterized a sodium-independent transport system which resembles system L. Since parietal cells amount to about 50% of total cell volume of the isolated gland in the rabbit [24,31], the data obtained in this study would suggest that amino acid transport occurs mainly through the basolateral membrane of the parietal cells. That the isolated oxyntic glands maintained their morpho-functional integrity was assessed from the following experimental findings (see Materials and Methods): (i) the glandular cells exhibited normal aspect by light and electron microscopy; (ii) the glandular cells rejected Trypan blue which is a characteristic of viable cells, and (iii) the relatively high uptake values of amino acids; e.g., the J_{max} of L-leucine influx was 13.9 nmol mg^{-1} s^{-1} (see Fig. 2) and that previously measured by us for L-alanine was 27.7 nmol mg^{-1} s^{-1} (recalculated from Ref. 17), suggests that the transport capacity of the basolateral membrane was preserved.

When the uptake of both tritium labelled L-leucine and L-phenylalanine was measured at different concentrations of their respective unlabelled analogues in a sodium-containing medium, significant inhibition was observed (Fig. 2). Removal of Na^+ from the medium did not affect the autoinhibition of the amino acid uptake. Instead, the uptake of L-leucine was inhibited at similar rates by L-isoleucine or L-phenylalanine independently of the presence or the absence of Na^+ in the medium (Fig. 3). Furthermore, short-chain neutral amino acids had no significant effect on the influx of L-leucine. These overall results suggest that a sodium-independent system which prefers branched-chain and aromatic dipolar amino acids is involved in the transport of L-leucine through the basolateral side of the oxyntic glands.

Corrections for nonsaturable components (see Figs. 2 and 4) were made in order to obtain the carrier-mediated uptake of the amino acids. In addition, any non-saturable component to substrate uptake was cancelled when a Hanes transformation of L-leucine (Fig. 5) and L-phenylalanine uptake values were performed. Therefore all kinetic constants measured in this study correspond to carrier-mediated transport of the amino acids.

The Michaelis-Menten analysis of L-leucine and L-phenylalanine uptake revealed that in both cases the influx was saturable and apparently mediated by a single entry system. The K_t obtained for the Na^+-independent carrier-mediated uptake of L-leucine (2.71 mM) in this preparation was considerably higher than that measured at the basolateral side of renal epithelial cell lines by Sepúlveda and Pearson [32]: $K_t = 0.06$ mM for the Na^+-independent transport of L-leucine. However, the K_t value reported in the present paper is of the same order as the value of 1.58 mM obtained for L-leucine transport across the luminal side of the rat blood-brain barrier [33] and of 2.2 mM in sacs of everted rat small intestine [34]. Markedly higher K_t values for L-leucine transport across the blood-tissue interface of the perfused dog stomach have been also obtained [16]. The observed K_t for the carrier-mediated uptake of L-phenylalanine (1.03 mM) is lower than the reported K_t values for the transport of L-phenylalanine in the perfused cat salivary gland [6], $K_t = 6.4$ mM, and in the perfused guinea-pig placenta [35], $K_t = 3.3$ mM at the maternal side and 11.9 mM at the fetal side. However, in these reports no correction for a non-saturable component of the amino acid uptake was made.

When the uptake of substrate analogues is mediated by a single carrier system the relative magnitude of the transport constant is particularly useful in predicting the extent to which analogues will compete with each other. The sodium-independent transport system which appears to be responsible for L-leucine uptake showed a marked preference for neutral amino acids with bulky side chains (Fig. 3). The observed K_i values for both L-isoleucine (2.62 mM) and L-phenylalanine (2.81 mM) of the carrier-mediated uptake of L-leucine (Fig. 4) are not very different to both K_t (2.71 mM) and K_i values obtained from self-inhibition experiments of L-leucine uptake: 3.2 mM from Dixon plot and 2.12 mM from Fig. 5. Instead K_i values of L-phenylalanine obtained from self-inhibition data: 2.4 mM (by Hanes plot as was described in the legend to Fig. 5) and 0.8 mM (by Dixon plot) are in the same order to the K_t (1.03 mM) measured for L-phenylalanine transport. These overall results suggest that branched chain and aromatic neutral amino acids share a Na^+-independent transport system which displays no substantial affinity difference for these substrates.

We conclude that the basolateral surface of rabbit oxyntic glands contains an amino acid transport system with some striking similarities to Christensen's L type which would operate in parallel with the previously described system A [17].

Acknowledgements

This work was supported by grant 2-.33.38 and 91.33.61-1 from the Dirección de Investigación, Universidad de Concepción. We are indebted to Dr. David Yudilevich and Karen Everet for their critical reading of the manuscript and to Mrs. Susana Rojas for her skilled technical assistance in some experiments and the typing of the text. The technical assistance of Mr. José Morales is also appreciated.

References

1 Boyd, C.A.R. and Perring, V.S. (1982) J. Physiol. 327, 53–64.
2 Mircheff, A.K., Van Os, C.H. and Wright, E.M. (1980) J. Membr. Biol. 52, 83–92.

3 Stevens, B.R., Kaunitz, J.D. and Wright, E.M. (1984) Annu. Rev. Physiol. 46, 417–433.
4 Hopfer, V. (1987) in Physiology of Gastrointestinal Tract (Johnson, L.R., ed.), pp. 1499–1526, Raven Press, New York.
5 Bustamante, J.C., Mann, G.E. and Yudilevich, D.L. (1981) J. Physiol. 313, 65–79.
6 Mann, G.E. and Yudilevich, D.L. (1984) J. Physiol. 347, 111–127.
7 Yudilevich, D.L. and Mann, G.E. (1982) Fed. Proc. 41, 3045–3053.
8 Yudilevich, D.L., Sobrevía, L. and Barros, L. (1991) in Cell Membrane Transport: Experimental Approaches and Methodologies (Yudilevich, D.L., Devés, R. Peran, S. and Cabantchik, Z.I., eds.) pp. 87–106, Plenum Press, London, New York.
9 Christensen, H.N. (1982) Physiol. Rev. 62, 1193–1233.
10 Christensen, H.N. (1990) Physiol. Rev. 70, 43–77.
11 Kilberg, M.S. (1982) J. Membr. Biol. 69, 1–12.
12 Bravo, I., Fuentes, O., Sobrevía, L. and Rojas, S. (1989) Med. Sci. Res. 17, 523–524.
13 Fuentes, O., Bravo, I. and Sobrevía, L. (1990) Med. Sci. Res. 18, 163–164.
14 Gutzlaff, D., Fuentes, V. and Bravo, I. (1992) Dental Res., in press.
15 Bravo, I., Rojas, S. and Cruz, M.A. (1986) J. Vet. Med. 33, 286–292.
16 Bravo, I., Fuentes, O., Pozo, M. and Rojas, S. (1988) Cell Mol. Biol. 34, 323–336.
17 Bravo, I. and Sobrevía, L. (1990) Biochim. Biophys. Acta 1029, 98–104.
18 Shotwell, M.A., Kilberg, M.S. and Oxender, D.L. (1983) Biochim. Biophys. Acta 737 267–284.
19 Christensen, H.N. and Kilberg, M.S. (1987) in Amino Acid Transport in Animal Cell (Yudilevich, D.L. and Boyd, C.A.R., eds.), pp. 1–46, Manchester University Press, Manchester.
20 Anderson, N.J. and Hanson, P.J. (1983) Biochem. J. 210, 451–455.
21 Ichichara, A., Noda, C. and Goto, M. (1975) Biochem. Biophys. Res. Commun. 63, 1313–1318.
22 Medina, V., Sobrevía, L. and Bravo, I. (1990) Arch. Biol. Med. Exp. 23, R-212.
23 Sobrevía, L. and Bravo, I. (1990) Arch. Biol. Med. Exp. 23, R332.
24 Berglindh, Th. and Öbrink, K.J. (1976) Acta Physiol. Scand. 96, 150–159.
25 Stevens, B.R., Ross, H.J. and Wright, E.M. (1982) J. Membr. Biol. 66, 213–225.
26 Le Cam, A. and Freychet, P. (1977) J. Biol. Chem. 252, 148–156.
27 Muflih, I.W. and Widdas, W.F. (1976) J. Physiol. 263, 101–114.
28 Sepúlveda, F.V. and Smith, M.W. (1978) J. Physiol. 282, 73–90.
29 Christensen, H.N. (1975) Curr. Top. Membr. Trans. 6, 227–258.
30 Berglindh, Th., Helander, H.F. and Öbrink, K.J. (1976) Acta Physiol. Scand. 97, 401–414.
31 Berglindh, Th., Dibona, D.K., Ito, S. and Sachs, S. (1980) Am. J. Physiol. 238, G165–G176.
32 Sepúlveda, F.V. and J.D. Pearson (1982) J. Cell Physiol. 112, 182–188.
33 Pardridge, W.M. (1983) Physiol. Rev. 63, 1481–1535.
34 Larsen, P.R., Ross, J.E. and Tapley, D.F. (1964) Biochim. Biophys. Acta 88, 570–577.
35 Eaton, B.M., Mann, G.E. and Yudilevich, D.L. (1982) J. Physiol. 328, 245–258.